KERATINOPHILIC AND CYCLOHEXIMIDE RESISTANT FUNGI IN SOILS OF SINAI GOVERNORATE, EGYPT

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ABSTRACT - Using human hair fragments as baits at 28°C, 44 keratinophilic cycloheximide resistant species and 2 varieties belonging to 16 genera were collected from 100 soil samples gathered from different places of Sinai Governorate (48 from cultivated area, 31 from desert and 21 from salt marshes). Numerous keratinophilic fungi were isolated namely: *Chrysosporium keratinophilum, C. tropicum, C. indicum, C.* state of *Thielavia sepedonium, C. asperatum, C. merdarium, C.* anamorph of *Arthroderma cuniculi, C. pannorum, C. queenslandicum, C. georgii, C. luteum, C.* state of *Arthroderma tuberculatum, Microsporum gypseum, M. cookei, Trichophyton mentagrophytes, T. longifusus, T. terrestre, Myceliophthora vellerea* and *Candida albicans*. Also several other saprophytic and cycloheximide resistant fungi were isolated.

RÉSUMÉ - 44 espèces de champignons kératinophiles résistant à l'actidione, et 2 variétés (= 16 genres), ont été isolées dans 100 échantillons de sols récoltés en différents lieux du Sinaï (48 sols cultivés, 31 sols désertiques, 21 sols de marais salant), en utilisant comme pièges des cheveux humains. Parmi ces champignons: *Chrysosporium keratinophilum, C. tropicum, C. indicum, C.* anam. de *Thielavia sepedonium, C. asperatum, C. merdarium, C.* anam. d' *Arthroderma cuniculi, C. pannorum, C. queenslandicum, C. georgii, C. luteum, C.* anam. d' *Arthroderma tuberculatum, Microsporum gypseum, M. cookei, Trichophyton mentagrophytes, T. longifusus, T. terrestre, Myceliophthora vellerea* et *Candida albicans*. Plusieurs autres champignons saprophytes résistant à l'actidione ont également été isolés.

KEY WORDS : keratinophilic fungi, cycloheximide resistant fungi, soil borne fungi.

INTRODUCTION

Studies on keratinophilic fungi are considerable significance and have been reported from soil of many countries all over the world (Ajello & Padhye, 1974; Caretta & al., 1977; Crozier, 1980; Mc Aleer, 1980; Sur & Ghosh, 1980; Calvo

& al., 1984) as well as from air in Italy (Della Franca & Caretta, 1984) and from a small pool (Mangiarotti & Caretta, 1984).

In Egypt, several surveys of these fungi were achieved (Abdel-Fattah & al., 1982; Abou-Gabal & Abd-Elrahiem, 1973; Bagy & Abdel-Hafez, 1985; Bagy, 1986; Moawad, 1969; Mostafa, 1977), but none of these studies were focused on Sinai soils. The present investigation aims to be an intensive study on the composition and frequency of occurrence of keratinophilic fungi and cycloheximide resistant fungi in soils of Sinai Governorate, Egypt.

The Sinai Peninsula covers an area of 61000km². It is triangular in shape and is separated geographically from Egypt by the Suez Canal and the Gulf of Suez. It is continuous with the Asiatic continent for a distance over 200km long between Rafa on the Mediterranean Sea and the head of the Gulf of Aqaba. The core of the Peninsula, situated near its southern end, consists of an intricate complex of high and very rugged igneous and metamorphic mountains. The northern two-thirds of the Peninsula is occupied by a great northward-draining limestone plateau which rises from the Mediterranean coast, extends southward, and terminates in a high escarpment on the northern flanks of the great igneous core. The central portion of the plateau surface forms a fairly open country draining to the Mediterranean by numerous effluents of Wadi El-Arish. The eastern and western edges of this plateau dissected by numerous narrow and deep rocky valleys draining to the Gulf of Aqaba and Suez. Beyond the northern parts of the peninsula and extending nearly to the Mediterranean coast, is a broad tract of sand dunes some of which attain heights of over 100m above sea level (Said, 1962). In salt marshes area the vegetation are mostly *Anthrocnemon glaucum, Cressa cretica, Halocnemon strobilaceum, Netraria retusa, Salicornia fruticosa* and *Zygophyllum album*. But, in desert locality the plant growth is a thin cover of *Alhagi maurorum, Hyoscyamus muticus, Opuntia retusa, Lygos raetum, Phragmites australis, Punica granatum, Tamarix nilotica* and *Zygophyllum coccineum*.

MATERIALS AND METHODS

One hundred soil samples were collected from different localities of Sinai Governorate representing cultivated area (48 samples), desert (31 samples) and salt marshes (21 samples), according to the method described by Johnson & al. (1959).

The soil samples were analysed chemically for the estimation of total soluble salts, elements (Ca, Mg, K and Na) and organic matter. A pH-meter (WGPYE model 290) was used for the determination of soil pH. The soil type was determined by the hydrometer method as described by Piper (1955) and most of samples are sandy.

Isolation of Keratinophilic Fungi:

The hair baiting technique was employed as recommended by Vanbreuseghem (1952), and as employed by Abdel-Fattah & al. (1982): 100g of soil were put in a sterile plate and a sufficient quantity of sterile distilled water (about 20-25% moisture content) was added and mixed thoroughly. Pieces of sterile human

hair were sprinkled on the surface of the moistened soil. Five plates were used for each sample; the plates were incubated at 28°C for 6-8 weeks, and the soil in plates was remoistened whenever necessary. The moulds which appeared on the baits were transferred to the surface of Sabouraud's dextrose agar medium (Moss & Mc Quown, 1969) which was supplemented with 20 units/ml of sodium penicillin, 40μg/ml of dihydrostreptomycin and 0.05% cycloheximide (Actidione). Before adding to the agar, the first 2 antibiotics were dissolved separately in sterile distilled water while the third was dissolved in methanol. The plates were incubated at 28°C for 3-4 weeks and the developing colonies were examined and identified.

RESULTS AND DISCUSSION

The soil samples tested were generally poor in organic matter content (0.08-2.14% of dry soil) and their contents in total soluble salts widely ranged between 0.1-18.1%, in Ca: 0.01-2.15mg, Mg: 0.01-0.63mg, K: 0.01-0.49mg, and Na: 0.01-2.19mg g dry soil. Abdel-Fattah (1973) found that the total soluble salts of Egyptian desert soils varies between 0.4-6.6%. The pH values of the soils tested were all in alkaline side (7.1-8.7), and this is in agreement with the pH values of cultivated soils collected from Delta area and Upper Egypt (Moubasher & Abdel-Hafez, 1978). However, Egyptian desert soils previously examined (Abdel-Fattah, 1973) were around neutrality (6.5-7.4).

Fourty-four keratinophilic and cycloheximide resistant species in addition to 2 varieties which belong to 16 genera were collected from 100 soil samples baited with human hair fragments at 28°C (Tabl. 1).

Chrysosporium was the most common genus, occurring in 69% of the samples. It was represented by 12 species of which *C. keratinophilum* was the most common species and was represented in 32% of the soil samples. *C. keratinophilum* emerged from 6% on children playgrounds sand samples (Bojanovsky & al., 1979); from 13.2% of soil samples in W. Germnay (Meissner & Qadripur, 1983); from 16.9% of soils of the Volcano Etna (Caretta & al.,1977); from 10% of the screened soil samples of swine habitats in U.S.A. (Ajello & al., 1964) and from 14% of soils of Spain (Calvo & al., 1984). In Egypt, this species constituted 0.4% only of the total isolates collected by Mostafa (1977). But Abdel-Fattah & al. (1982) recovered this species from Egyptian soils baited with human (10% of the soil samples tested) and animal (20%) hair.

C. tropicum was the second most frequent fungal species and was encountered in 18% of the soil samples tested. It was dominant species in Italy soils (24.5% of the samples) as recorded by Todaro (1978). It was also represented in 20.8 and 12.4% of the soil samples in Marrakesh and Casablanca (Jana & al., 1979). In India, *C. tropicum* occurred in 18% of the soil samples tested (Sur & Ghosh, 1980); in Galapagos Islands in 5.3% (Ajello & Padhye, 1974); in Chilean Andes in 3.9% (Piontelli & Caretta, 1974); in soil of Volcano Etna, 20.5% (Caretta & al., 1977); and Spain in 24% (Calvo & al., 1984). In Egypt, *C. tropicum* was the most common fungal species recovered by baiting, comprising 36.6% of the total fungal isolates (Mostafa, 1977). Moawad (1969) obtained this species only from soil samples taken from El-Fayum Governorate. No other chrysosporia were iso-

lated by him. Abdel-Fattah & al. (1982) isolated *C. tropicum* in 18.5 and 11.4% of the soil samples collected from Assiut Governorate and baited with human and animal hair, respectively.

Table 1: numbers of cases of isolation, percentage frequencies and occurrence remarks of fungal genera and species recovered from soils baited with human hair and incubated at 28°C.

Tableau 1: genres et espèces de champignons isolés à partir de sols, en utilisant comme pièges des cheveux humains. Incubation à 28°C.

Genera and species	NCI and % F	OR
Chrysosporium	69	H
C. keratinophilum (Frey) Carmichael	32	M
C. tropicum Carmichael	18	L
C. indicum	17	L
C. state of *Thielavia sepedonium* Emmons	15	L
C. asperatum Carmichael	8	R
C. merdarium (Link ex Grev.) Carmichael	7	R
C. anamorph of *Arthroderma cuniculi* Dawson	6	R
C. pannorum (Link) Hughes	4	R
C. queenslandicum Apinis & Rees	4	R
C. georgii (Varsavsky & Ajello) Van Oorschot	3	R
C. luteum Constantin	3	R
C. state of *Arthroderma tuberculatum* Kuehn	2	R
Aspergillus	37	M
A. flavus Link	14	L
A. rugulosus Thom & Raper	12	R
A. nidulans (Eidam) Wint.	10	R
A. fumigatus Fresenius	7	R
A. nidulans var. *dentatus* Sandhu & Sandhu	5	R
A. tamarii Kita	4	R
A. nidulans var. *latus* Thom & Church	2	R
A. terreus Thom	2	R
A. versicolor (Vuill.) Tirab.	2	R
A. sydowi (Bain. & Sart) Thom & Church	1	R
Chaetomium	21	L
C. globosum Kunze ex Fr.	13	L
C. olivaceum Cooke & Ellis	9	R
C. trigonosporum (Marchal) Chivers	2	R
Microsporum	13	L
M. gypseum (Bodin) Guiart & Grigorakis	12	R
M. cookei Ajello	1	R
Scopulariopsis brevicaulis (Sacc.) Bainier	12	R
Trichophyton	10	R
T. mentagrophytes (Robin) Blanchard	6	R
T. longifusus (Florian & Galgoczy) Ajello	4	R
T. terrestre Durie & Frey	1	R
Fusarium	9	R
F. solani (Mart.) Sacc.	6	R
F. acuminatum Ellis & Everhart	2	R
F. oxysporum Schlecht.	1	R
Acremonium	6	R

A. nutilum W. Gams	5	R
A. furcatum F. & V. Moreau ex W. Gams	3	R
Penicillium	6	R
P. nigricans (Bainier) Thom	3	R
P. funiculosum Thom	2	R
P. variabile Sopp	1	R
Sepedonium chrysospermum (Bull.) Link	5	R
Geotrichum candidum Link	3	R
Myceliophthora vellerea (Sacc. & Speg.) Van Oorschot	3	R
Candida albicans (Robin) Berkhout	1	R
Cunninghamella elegans Lender	1	R
Paecilomyces lilacinus (Thom) Samson	1	R
Syncephalastrum racemosum (Cohn) Schroeter	1	R

NCI: number of cases of isolation (out of 100 soil samples). %F: percentage frequency of occurrence (calculated per 100 samples). OR: occurrence remarks: H: high occurrence, isolated more than 49 cases (out of 100 soil samples), M: moderate occurrence, from 25 to 49 cases, L: low occurrence, from 13 to 24 cases, R: rare occurrence, less than 13 cases.

C. indicum was the third most frequent fungal species and was represented in 17% of the soil samples, whereas it was the most frequent species in cultivated soils collected from Upper Egypt (Abdel-Fattah & al., 1982), but it was less frequent in Egyptian soils tested by Mostafa (1977), as well as from wild sparrow feather and nest (Khalil, 1982). In India, it emerged from 31.3% of the soil samples (Sur & Ghosh, 1980); in mountainous localities in the Chilean Andes, from 19.8% (Piontelli & Caretta, 1974); in Galapagos Islands from 2.6% (Ajello & Padhye, 1974); and Spain, from 4% (Calvo & al., 1984) of the soil samples tested.

Chrysosporium state of *Thielavia sepedonium* was emerged in 15% of the samples tested. Bagy & Abdel-Hafez (1985) isolated the previous species from 5 and 8% of the samples of camel and goat hairs, respectively. *C. asperatum*, *C. merdarium*, *C.* anamorph of *Arthroderma cuniculi*, *C. pannorum*, *C. queenslandicum*, *C. georgii*, *C. luteum* and *C.* state of *Arthroderma tuberculatum* were recovered in rare frequency.

Aspergillus was the second most frequent genus and was encountered in 37% of the samples tested. From the genus 8 species and 2 varieties were collected of which *A. flavus*, *A. rugulosus*, *A. nidulans* and *A. fumigatus* were the most common. The remaining *Aspergillus* species were scarcely recovered and these were *A. nidulans* var. *dentatus*, *A. tamarii*, *A. nidulans* var. *latus*, *A. terreus*, *A. versicolor* and *A. sydowi*. Aspergillosis due to *A. fumigatus* and *A. flavus* has a world-wide distribution (Frey & al., 1979). *A. flavus*, *A. fumigatus* and *A. nidulans* were present in cases of onychomycosis in Colombia (Velez & Diaz, 1985); *A. flavus*, *A. fumigatus*, *A. nidulans* and *A. sydowi* from hair of large mammals in Egypt (Bagy, 1986); and *A. candidus*, *A. sydowi*, *A. terreus* and *A. versicolor* were isolated from nails by English & Atkinson (1974).

Chaetomium occupied the third place with regard to the number of cases of isolation of fungal genera and it recovered from 21% of the samples examined. Three species of *Chaetomium* were isolated and these were *C. globosum* (13%), *C. olivaceum* (9%) and *C. trigonosporum* (2%). Guarro & al. (1981) isolated *C.*

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